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CONT.

found in these proteins. In Figure 3 there is shown a collinear amino acid sequence alignment of mouse Gl $\alpha$  (mCASH $\alpha$ ) (SEQ ID NO:5), human Gl $\alpha$  (hCASH $\alpha$ ) (SEQ ID NO:2) and Gl $\beta$  (hCASH $\beta$ ) (SEQ ID NO:4), CASP-8 (MACH/FLICE1/Mch5) (SEQ ID NO:6), CASP-10 (Mch4/FLICE2) (SEQ ID NO:7), CASP-3 (CPP32/Apopain/Yama) (SEQ ID NO:8) and CASP-1 (ICE) (SEQ ID NO:9). CASP-1 and CASP-3 are shown without their prodomain regions. Amino acid residues are numbered to the right of each sequence. Dotted lines indicate gaps in the sequence to allow optimal alignment. The 'death domain' modules (DED) are shaded. Amino acids that are identical in more than three of the proteins shown are boxed. Within the region of protease homology, amino acids aligned with CASP-1 residues that were implicated in catalytic activity by X-ray crystallography are denoted as follows : The residues putatively involved in catalysis, corresponding to His237 and Cys285 in CASP-1, are darkly shaded and marked by closed circles below the alignment. The residues constituting the binding pocket for the carboxylate side chain of the P1 Asp, corresponding to Arg179, Gln 238, Arg341 and Ser347 in CASP-1, are less heavily shaded and marked by open circles. Known and suggested Asp-X cleavage sites and the potential site of cleavage found at a similar location in Gl (CASH) are shaded. Horizontal arrows indicate the N- and C- terminal ends of the small and large subunits of the CASP-1. The C-termini of the proteins are denoted by asterisks.--

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Please replace the paragraph beginning at page 45, line 21, with the following rewritten paragraph:

E2  
--A non-limiting example of how peptide inhibitors of the G1 proteases would be designed and screened is based on previous studies on peptide inhibitors of ICE or ICE-like proteases, the substrate specificity of ICE and strategies for epitope analysis using peptide synthesis. The minimum requirement for efficient cleavage of peptide by ICE was found to involve four amino acids to the left of the cleavage site with a strong preference for aspartic acid in the P<sub>1</sub> position and with methylamine being sufficient to the right of the P<sub>1</sub> position (Sleath et al., 1990; Howard et al., 1991; Thornberry et al., 1992). Furthermore, the fluorogenic substrate peptide (a tetrapeptide), acetyl-Asp-Glu-Val-Asp-a-(4-methyl-coumaryl-7-amide) abbreviated Ac-DEVD-AMC (SEQ ID NO:10), corresponds to a sequence in poly (ADP-ribose) polymerase (PARP) found to be cleaved in cells shortly after FAS-R stimulation, as well as other apoptotic processes (Kaufmann, 1989; Kaufmann et al., 1993; Lazebnik et al., 1994), and is cleaved effectively by CPP32 (a member of the CED3/ICE protease family) and MACH proteases (and likewise also possibly by G1 proteases).--

Please replace the paragraph beginning at page 46, line 13, with the following rewritten paragraph:

E3  
--Since it may be advantageous to design peptide inhibitors that selectively inhibit G1 proteases without interfering with physiological cell death processes in which

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other members of the CED3/ICE family of proteases are involved, the pool of peptides binding to G1 proteases in an assay such as the one described above can be further synthesized as a fluorogenic substrate peptide to test for selective cleavage by G1 proteases without being cleaved by other CED3/ICE proteases. Peptides which are determined to be selectively cleaved by the G1 proteases, can then be modified to enhance cell permeability and inhibit the cell death activity of G1 either reversibly or irreversibly. Thornberry et al. (1994) reported that a tetrapeptide (acyloxy) methyl ketone Ac-Tyr-Val-Ala-Asp-CH<sub>2</sub>OC (O)-[2,6-(CF<sub>3</sub>)<sub>2</sub>] Ph (SEQ ID NO:11) was a potent inactivator of ICE. Similarly, Milligan et al. (1995) reported that tetrapeptide inhibitors having a chloromethylketone (irreversibly) or aldehyde (reversibly) groups inhibited ICE. In addition, a benzyloxycarboxyl-Asp-CH<sub>2</sub>OC (O) -2,6-dichlorobenzene (DCB) was shown to inhibit ICE (Mashima et al., 1995). Accordingly, tetrapeptides that selectively bind to G1 proteases can be modified with, for example, an aldehyde group, chloromethylketone, (acyloxy) methyl ketone or a CH<sub>2</sub>OC (O)-DCB group to create a peptide inhibitor of G1 protease activity.--

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Please replace the paragraph beginning at page 81, line 7, with the following rewritten paragraph:

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--Exploring the nature of this size heterogeneity, cDNA libraries were screened for transcripts that hybridize with the MACH31 cDNA probe. MACH $\alpha$ 1 and MACH $\alpha$ 2 were cloned

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from a Charon BS cDNA library derived from the mRNA of human thymus. The library was screened under stringent conditions with a MACH $\beta$ 1 cDNA probe, labeled using a random-priming kit (Boehringer Mannheim). The other MACH isoforms were cloned by RT-PCR, performed on total RNA from Raji (MACH $\alpha$ 1,  $\alpha$ 2,  $\alpha$ 3,  $\beta$ 3 and  $\beta$ 4) and Daudi (MACH $\alpha$ 2,  $\beta$ 2,  $\beta$ 3,  $\beta$ 4, and  $\beta$ 5) human lymphoblastoid cells. Reverse transcriptase reaction was performed with an oligo-dT adapter primer (5'-GACTCGAGTCTAGAGTCGAC(T)<sub>17</sub>-3') (SEQ ID NO:12) and the SuperScript II reverse transcriptase (GIBCO-BRL), used according to the manufacturer's instructions. The first round of PCR was performed with the Expand Long Template PCR System (Boehringer Mannheim) using the following sense and antisense primers: 5'-AAGTGAGCAGATCAGAATTGAG-3' (SEQ ID NO:13), corresponding to nucleotides 530-551 of the MACH $\beta$ 1 cDNA, and 5'-GACTCGAGTCTAGAGTCGAC-3' (SEQ ID NO:14), respectively. The second round was performed with Vent polymerase (NEB) using the following sense and antisense nested primers: 5'-GAGGATCCCCAAATGCAAACCTGGATGATGAC-3' (SEQ ID NO:15) and 5'-GCCACCAGCTAAAAACATTCTCAA-3' (SEQ ID NO:16), derived from the sequence of MACH $\beta$ 1 cDNA, respectively. To confirm that MACH $\beta$ 3 and MACH $\beta$ 4 have initiation codons, a more 5' sequence of these isoforms from the RNA of Raji cells was cloned. The RT-PCR reaction, performed using the oligo-dT adapter primer as described above, was followed by two rounds of PCR (with Vent polymerase (NEB)) using the following sense and antisense oligonucleotides: 5'-TTGGATCCAGATGGACTTCAGCAGAAATCTT-3' (SEQ

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CONT.

ID NO:17) and 5'-ATTCTCAAACCCTGCATCCAAGTG-3' (SEQ ID NO:18), derived from the sequence of MACH $\beta$ 1. The latter oligonucleotide is specific to the  $\beta$ -isoforms. Among the clones obtained in this way, those found to contain the nucleotides encoding for the amino acids of 'block 2' (whose presence distinguishes MACH $\beta$ 3 and MACH $\beta$ 4 from MACH $\beta$ 1 and MACH $\beta$ 2) were fully sequenced. Nucleotide sequences in all cloned isoforms were determined in both directions by the dideoxy-chain termination method. Only partial cDNA clones of MACH $\alpha$ 3 and MACH $\beta$ 2 were obtained. This screening revealed the existence of multiple isoforms of MACH MACH. The amino acid sequences of seven of these isoforms were studied in detail. The results are illustrated diagrammatically and exemplified in the above co-owned co-pending applications, particularly PCT/US96/10521 and IL 117932, where the amino acid sequences of three of the isoforms are compared with known homologs.--

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Please replace the paragraph beginning at page 85, line 3, with the following rewritten paragraph:

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E5

--To find out if the CED3/ICE homology region in MACH $\alpha$  possesses proteolytic activity, applicants expressed the region that extends from the potential cleavage site upstream to this region, between Asp216 and Ser217, till the C terminus of the protein in bacteria, as a GST fusion protein. The bacterial lysates were examined for ability to cleave fluorogenic peptide substrates, shown before to be cleaved by other CED3/ICE homologs. Two substrate peptides were used:

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The first, Acetyl-Asp-Glu-Val-Asp-a-(4-Methyl-Coumaryl-7-Amide) (AC-DEVD-AMC) (SEQ ID NO:10), corresponds to a sequence in poly (ADP-ribose) polymerase (PARP), a nuclear protein found to be cleaved in cells shortly after FAS-R stimulation (Tewari et al., 1995b), as well as in other apoptotic processes (Kaufmann, 1989; Kaufmann et al., 1993; Lazebnik et al., 1994). This fluorogenic substrate is cleaved effectively by CPP32. The second fluorogenic substrate, Acetyl-Tyr-Val-Ala-Asp-AMC (Ac-YVAD-AMC) (SEQ ID NO:11), corresponds to a substrate site for ICE in the IL-1 $\beta$  precursor. This fluorogenic substrate is cleaved by ICE. Lysates of bacteria expressing the CED3/ICE homology region in MACH $\alpha$ 1 cleaved effectively the PARP sequence-derived fluorogenic substrate. They had no measurable proteolytic activity, though, against the IL-1 $\beta$ -precursor sequence-derived fluorogenic substrate (controls), Ac-YVAD-AMC, which is an ICE cleavage site in IL-1 $\beta$  precursor (Thornberry et al, 1992). The proteolytic activity was blocked by iodoacetic acid (5 mM), confirming that it is mediated by a thiol protease. No cleavage was observed with lysates containing the GST-fused MACH CED3/ICE-homology region in which the catalytic cysteine residue Cys<sub>360</sub> was replaced by Ser. Also, lysates from bacteria that expressed the full-length MACH $\alpha$ 1 protein as a GST-fusion protein did not cleave Ac-DEVD-AMC, probably because of the absence of bacterial enzymes capable of processing the full-length molecule. Nor did cleavage occur

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with lysates containing either of the two potential cleavage products of the CED3/ICE homology region.--

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Please replace the paragraph beginning at page 93, line 27, with the following rewritten paragraph:

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E6  
--A preliminary sequence of one such G1 isoform, a G1 splice variant, is depicted in Fig. 1A+B, in which Fig. 1A is the nucleotide sequence and Fig. 1B is the deduced amino acid sequence of an ORF starting from ATG (nucleotide No. 482) and terminating at TAA (nucleotide 1921), these start and terminator nucleotides being indicated by asterises (\*) in the nucleotide sequence. The G1 splice variant of Fig. 1 has also been putatively designated 'G1 $\alpha$ '.--

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Please replace the paragraph beginning at page 95, line 14, with the following rewritten paragraph:

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E7  
--Based on the nucleotide sequence of an EST clone found to correspond to the mouse homologue of part of the 'death domain' (DED) region in G1, the cDNAs of both the mouse CASH $\alpha$  and CASH $\beta$  splice variants were cloned from mouse liver mRNA by RT-PCR. An EST clone (GenBank accession no. AA198928) was identified as the mouse homologue of part of the DED region in G1. Based on this sequence the mouse G1 $\alpha$  (CASH $\alpha$ ) and G1 $\beta$  (CASH $\beta$ ) splice variants from mouse liver mRNA were cloned by RT-PCR. The reverse transcriptase reaction was performed with an oligo-dT adapter primer (5'-GACTCGAGTCTAGAGTCGAC(T)<sub>17</sub>-3') (SEQ ID NO:12) and the AMV

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reverse transcriptase (Promega), used according to the manufacturer's instructions. The first round of PCR was carried out with the Expand Long Template PCR System (Boehringer Mannheim) using the following sense and antisense primers : 5'-GGCTTCTCGTGGTTCCCAGAGC-3' (SEQ ID NO:19), and 5'-GACTCGAGTCTAGAGTCGAC-3' (base pairs 1-20 of SEQ ID NO:12) (adapter) respectively. The second round was performed with Vent polymerase (NEB) using the nested sense primer : 5'-TGCTCTTCCTGTGTAGAGATG-3' (SEQ ID NO:20), and adapter.--

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Please substitute the attached paper copy Sequence Listing section, pages 1-17, for the one previously submitted on June 27, 2000.